GAS CHROMATOGRAPHY

- Chromatography involves a sample (or sample extract) being dissolved in a mobile phase (which may be a gas, a liquid or a supercritical fluid).

- The mobile phase is then forced through an immobile, immiscible stationary phase.

- The phases are chosen such that components of the sample have different solubilities in each phase. A component which is quite soluble in the stationary phase will take longer to travel through it than a component which is not very soluble in the stationary phase but very soluble in the mobile phase.

- As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase.

Gas Chromatography

Gas Chromatography is a **separation method** and is mainly applied on an analytical scale (sample amounts of less than 10^{-6} g =1µg/component).
Compounds must be sufficiently volatile and should evaporate without decomposition (gases, liquids and solids).

GC may be used for **identification** and **quantification** of the constituents of a mixture. **Identification** can be improved by coupling of the gas chromatograph to a specific detector, e.g. a mass spectrometer **GC/MS**.

GC may also be used as an **isolation** method for pure single compounds out of a complex mixture **(Preparative GC).**

The 4 main principles of chromatography:

Stationary Phase	Mobile Phase
Liquid (L)	Liquid (L)
Gaseous (g)	Solid (S)

GSC : Adsorption chromatography

GLC: Partition chromatography

Distribution of analytes between phases

$$A_{mobile} \rightleftharpoons A_{stationary}$$

The equilibrium constant, K, is termed the partition coefficient; defined as the molar concentration of analyte in the stationary phase divided by the molar concentration of the analyte in the mobile phase.

PARAMEERS IMPORTAN IN CHOOSING THE CONDIIONS OF SEPARATION

1- Retention factor (k`)

= Capacity factor

2- Selectivity factor (α)

3- Resolution

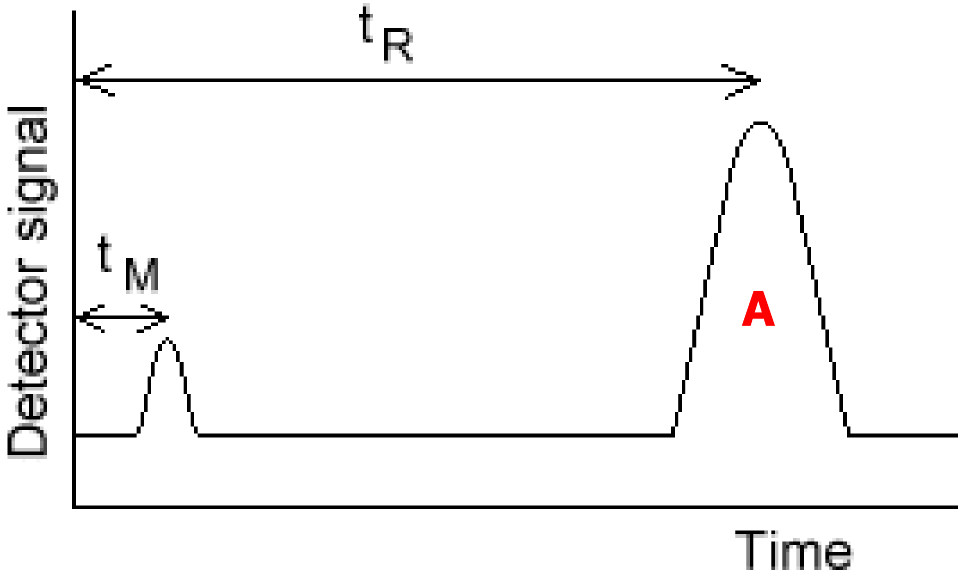
1-Retention factor, k' = Capacity factor

<u>The retention factor, k',</u> is often used to describe the migration rate of an analyte on a column

<u>The retention factor</u> for analyte A is defined as;

= k`(A) = t R - tM / tM **IF< 1** **If > 20** **([K' rang: 10>K'>2].**

$$k'(A) = \frac{t_R - t_M}{t_M}$$



t_R Retention time: The time between **sample** injection and an analyte peak reaching a detector at the end of the column

Each analyte in a sample will have a different retention time.

t_M The time taken for the **mobile** phase to pass through the column

2. Selectivity factor, α (SEPARATION)

selectivity factor, α describes the separation of two species (A and B) on the column.

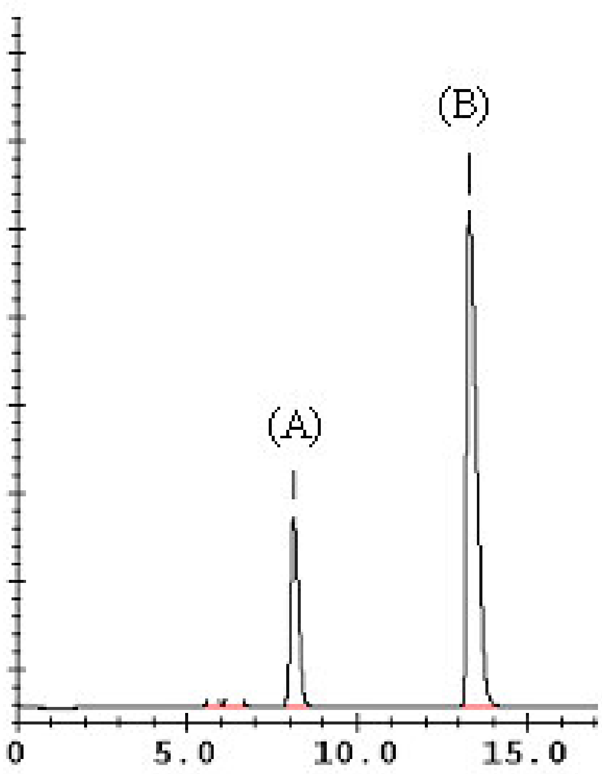
α = k 'B / k 'A = tRB – tM / tRA – tM

α = tRB / tRA

$\alpha \neq 1$

(if α = 1, this means that the two peaks are overlapped)

When calculating the selectivity factor, species A elutes faster than species B. The selectivity factor is always greater than one.



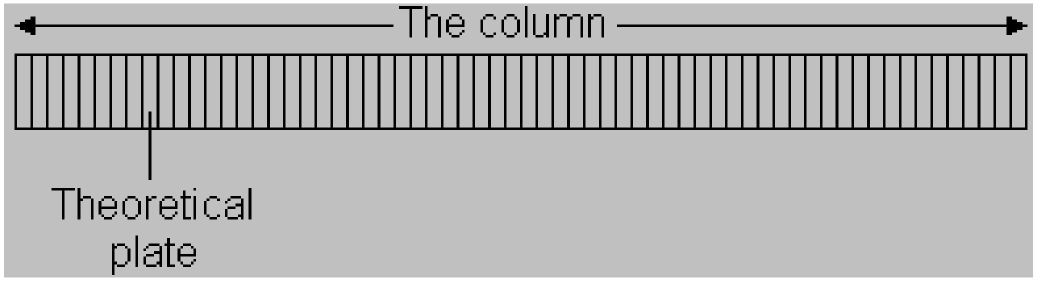
Band broadening and column efficiency

To obtain optimal separations, sharp, symmetrical chromatographic peaks must be obtained. This means that <u>band broadening</u> must be <u>limited</u>. It is also beneficial to measure the <u>efficiency</u> of the column.

<u>The Theoretical Plate Model of Chromatography</u>

<u>column efficiency (N)</u>

(The <u>efficiency</u> in all chromatographic techniques is expressed qualitatively as <u>the number of theoretical plates, N</u>, of the column)



If the length of the column is L, then the HETP (height equivalent to a theoretical plate) is

HETP = L / N

The number of theoretical plates that a real column possesses can be found by examining a chromatographic peak after elution;

$$N = \frac{5.55\, t_R^{\,2}}{w_{1/2}^{\,2}}$$

where $w_{1/2}$ is the peak width at half-height.

As can be seen from this equation, columns behave as if they have different numbers of plates for different solutes in a mixture.

($\downarrow$ HETP $\rightarrow \uparrow$ N $\rightarrow \uparrow$ Efficiency)

The Rate Theory of Chromatography

If we consider the various mechanisms which contribute to band broadening, we arrive at the Van Deemter equation for plate height;

$$HETP = A + B / u + C u$$

where u is the average velocity of the mobile phase. A, B, and C are factors which contribute to band broadening.

$HETP = 2\lambda dp \quad + \quad 2\gamma Dg/u \quad + 8/\pi 2 * K'/[1+K']2 * df2/DL * u$

$A \quad + \quad B/u \quad + \quad C*u$

Solid support — mobile gas — Liquid stationary phase

A=Eddy diffusion — B=Longitudinal diffusion — C=Resistance to mass transfer

Solid support: λ Irregularity factor of solid support (packing problem)- dp Particle diameter of solid support.

Mobile gas: γ Tortousity factor of mobile phase – Dg Diffusion coefficient in mobile phase- U Velocity of mobile gas (flow rate)

Stationary phase: K' Capacity factor- Df Liquid film thickness of stationary phase- DL Diffusion coefficient in liquid stationary phase.

A - Eddy diffusion (Multipath Effect) [Solid support]

Solute molecules will take different paths through the stationary phase at random. This will cause broadening of the solute band, because different paths are of different lengths **(as dp ↓ . λ ↓)**

B - Longitudinal diffusion (Molecular diffusion term) [mobile gas]

The concentration of analyte is less at the edges of the band than at the centre. Analyte diffuses out from the centre to the edges. If the velocity of the mobile phase is **high** then the analyte **spends less time** on the column, which decreases the effects of longitudinal diffusion **(↓DG by the use of viscous mobile phase (N_2), for fast separation (He or H_2). (also γ ↓as dp ↓ so ↓dp ↑ u)**

C - Resistance to mass transfer [Liquid stationary phase]

The analyte takes a certain amount of time to equilibrate between the stationary and mobile phase. If the velocity of the mobile phase is **high**, and the analyte has a **strong affinity** for the stationary phase, then the analyte in the mobile phase will move ahead of the analyte in the stationary phase. The band of analyte is broadened. **The higher the velocity of mobile phase, the worse the broadening becomes. (to decrease C try to ↑K', ↑ DL, ↓ df, ↓ u)**

The effects described earlier indicated that:

- **Eddy diffusion** can be minimised by reducing the diameter of the support consistent with maintaining a uniform packing structure

- **Longitudinal diffusion** is essentially eliminated at high mobile phase velocity.

- **Mass transfer**, although made worse by increasing the mobile phase velocity, can be minimised in the mobile phase by reducing the diameter of the support and /or eliminating long, narrow pores within the particles. In the stationary phase, the mass transfer is minimised by using, where possible, phase of low viscosity, thinly coated on the support material.

Eddy diffusion

From this Figure

Sample band broadening due eddy diffusion

(A) Initial concentration profile

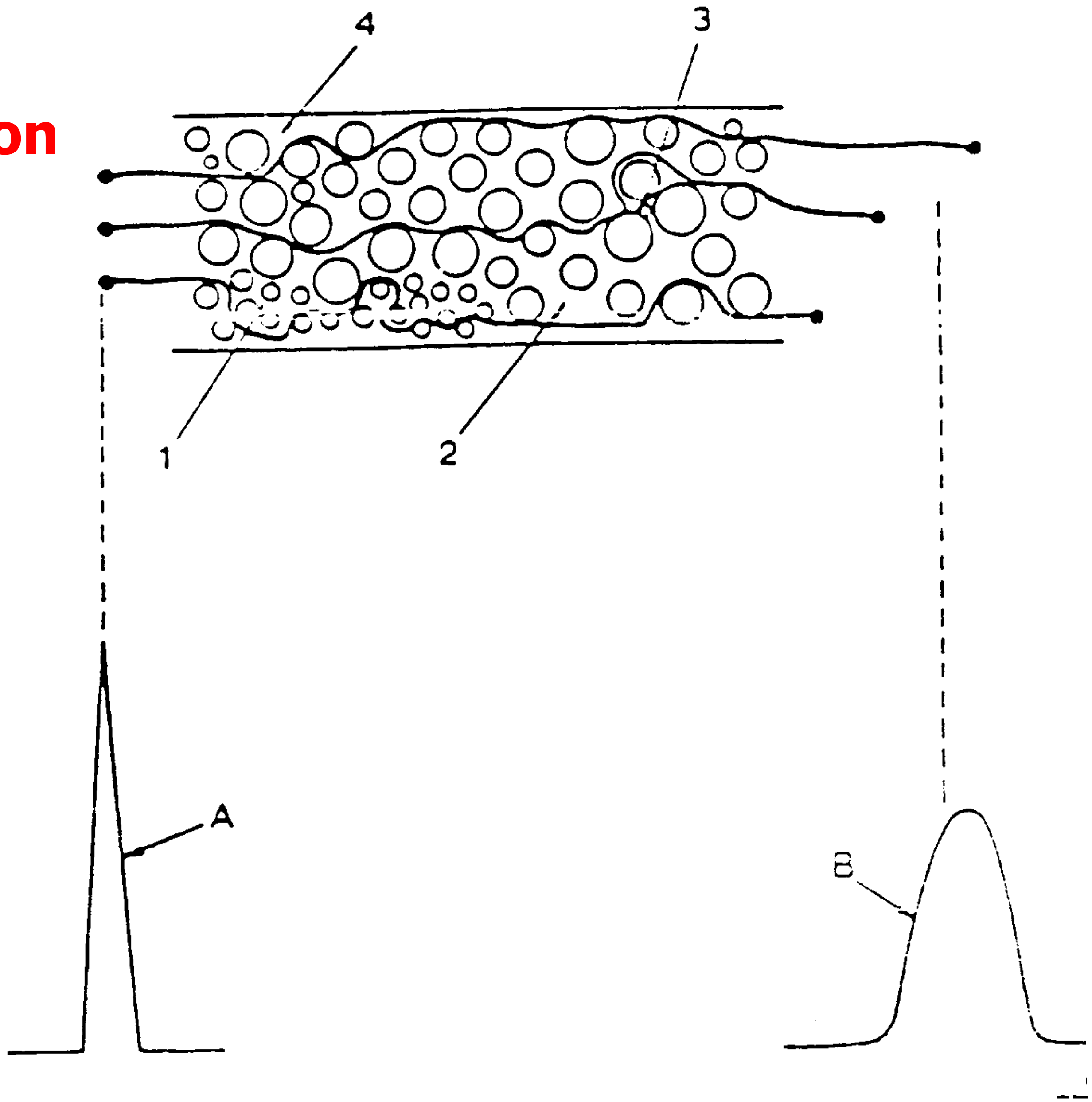
(B) Final concentration profile

1- Fine particles

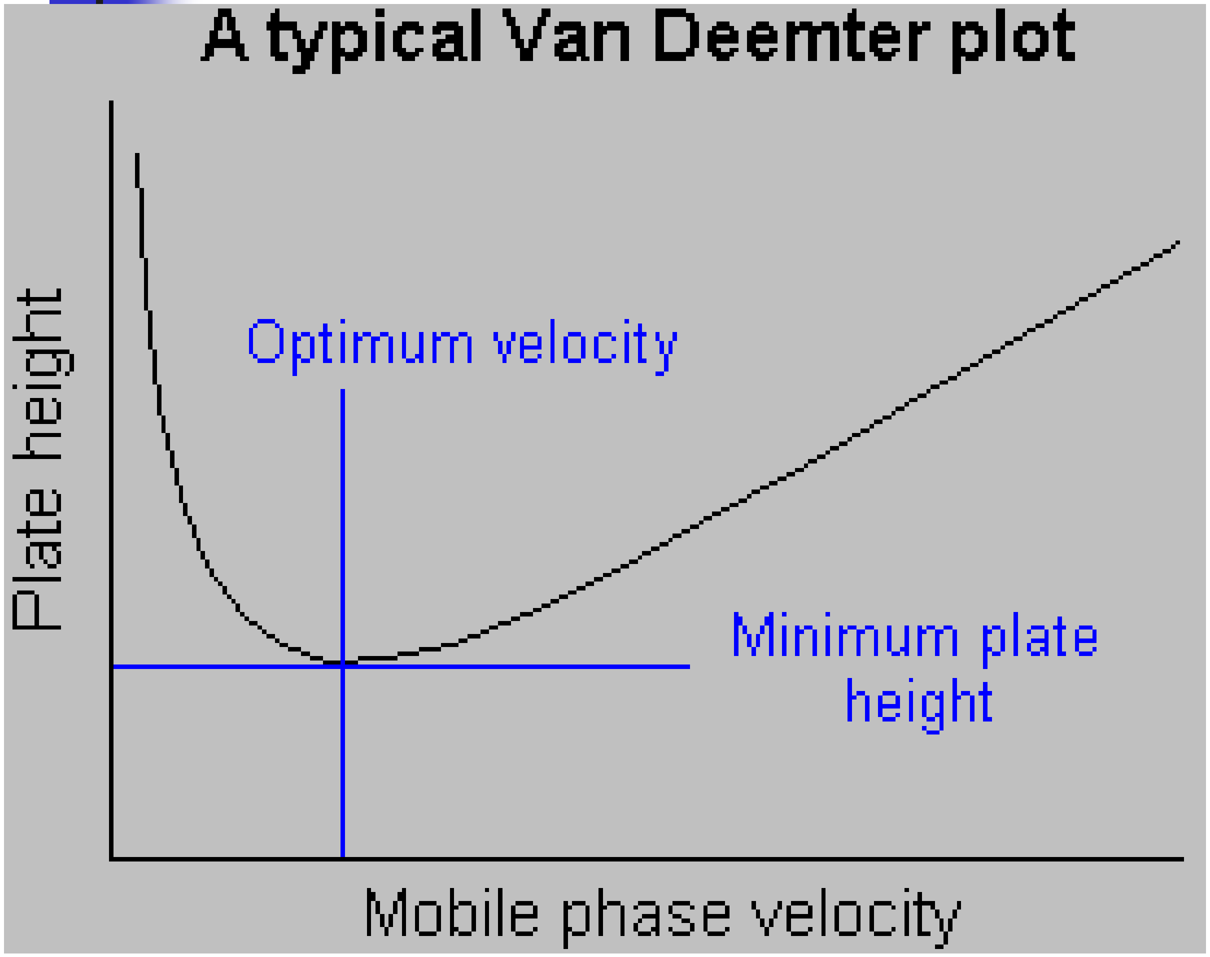
2- Coarse particles

3- agglomerated particles

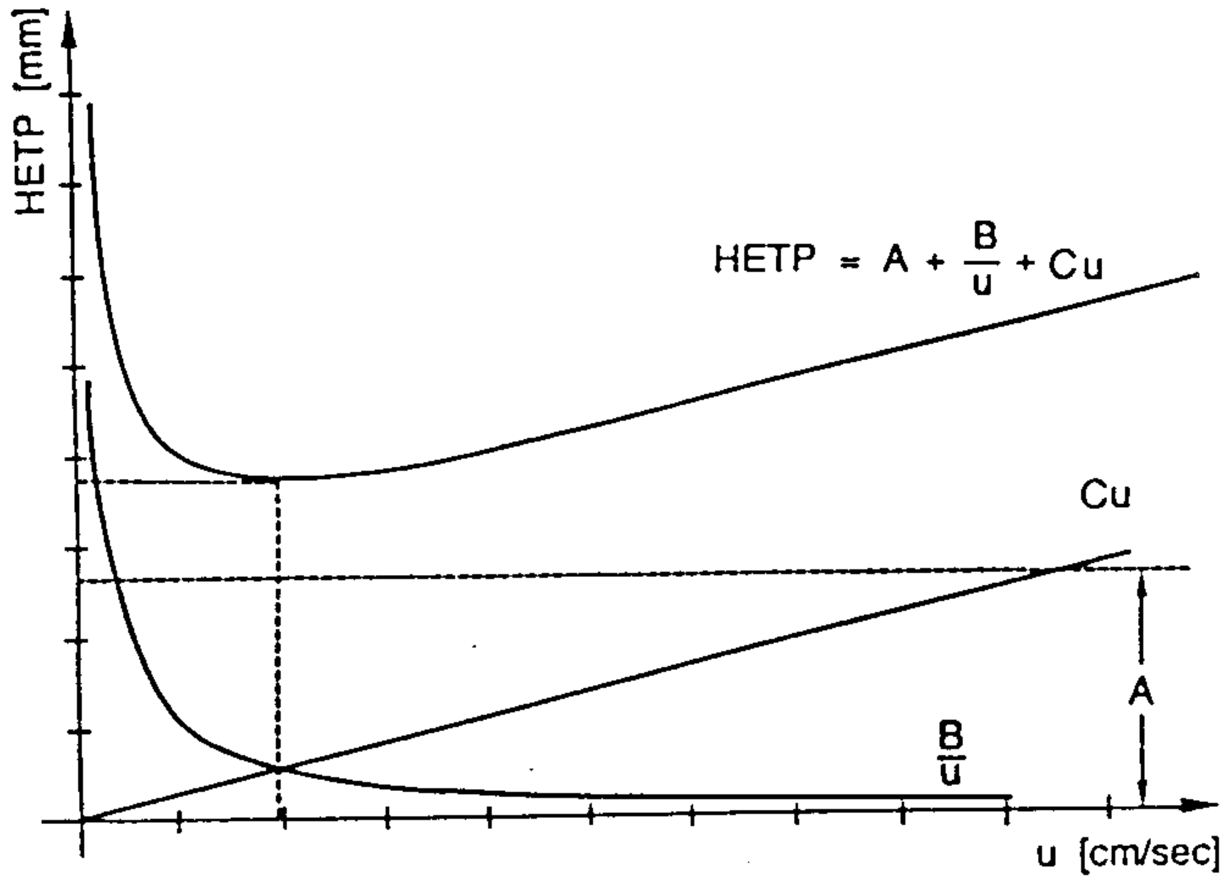
4-Low density of packing near column wall



Van Deemter plots



OGV: **<u>The point at which the column acquires the lower HEPT.</u>**
The operating gas velocity should be slightly higher than the OGV to control any disturbance or obstruction of gas flow

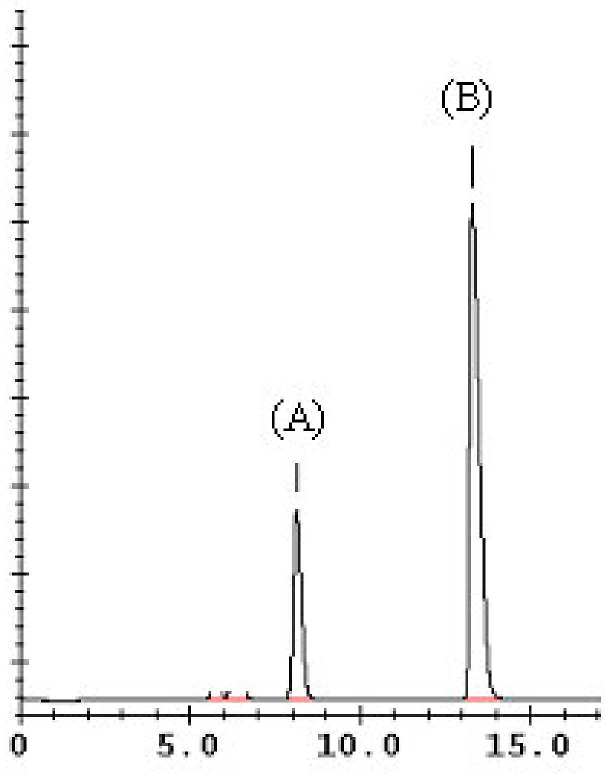


Influence of Carrier Gas Flow Velocity, u, on the Band Broadening During the Chromatographic Separation (Efficiency) Measured as HETP.

3. Resolution

Resolution, takes in account peak widths (Baseline resolution).
The resolution of two species, A and B, is defined as

$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$



Baseline resolution is achieved when R = 1.5
W: peak half-widths. (W ↑, R ↓, Column efficiency ↓)

Peak symmetry factor : (0.9 – 1.1) Ideal = 1
Non- uniform shapes are caused by a number of factors

1. Kinetic effect
2. Non-linearity of the partition isotherm
3. <u>Column overloading</u>
4. <u>Poor injection techniques</u>.

It is useful to relate the <u>resolution</u> to the <u>number of plates</u> in the column, the <u>selectivity factor</u> and the <u>retention factors</u> of the two solutes;

$$R = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{1 + k'_B}{k'_B}\right)$$

Efficiency Selectivity Capacity

To obtain high resolution,

The three terms must be maximised.

Efficiency : **An increase in N**, the number of theoretical plates, by lengthening the column leads to an increase in retention time and increased band broadening - which may not be desirable. Instead, to increase the number of plates, the height equivalent to a theoretical plate can be reduced by **reducing the size of the stationary phase particles.**

Capacity : It is often found that by controlling the **capacity factor, k',** separations can be greatly improved. This can be achieved by **changing the temperature** (in Gas Chromatography) or the composition of the mobile phase (in Liquid Chromatography)

The selectivity factor, α,

can also be manipulated to improve separations. When α is close to unity, optimising k' and increasing N is not sufficient to give good separation in a reasonable time. In these cases, k' is optimised first, and then α is increased by one of the following procedures:

1.Changing mobile phase composition
2.Changing column temperature
3.Changing composition of stationary phase
4.Using special chemical effects (such as incorporating a species which complexes with one of the solutes into the stationary phase)

Finally, Resolution of two compounds is sufficiently large

$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

•If the peak width is small compared to the retention time difference ΔtR.

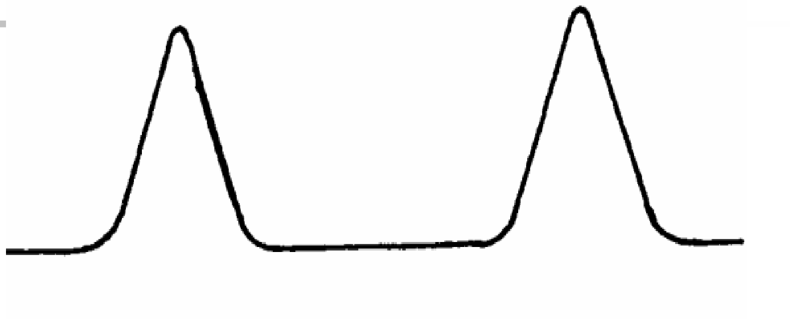
•Narrow peaks can only be achieved if the plate number of the column is sufficiently high.

•ΔtR is also related to the length of the column and the amount of stationary phase of in the column.

Only symmetrical peaks should be used for measuring resolution. Obviously resolution can also be achieved by column with low efficiency but high selectivity

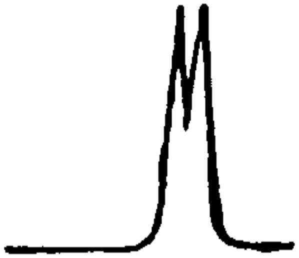
The Following figure shows the relation between efficiency, and selectivity on resolution of chromatographic colu

Good resolution through high efficiency



Good resolution through high selectivity

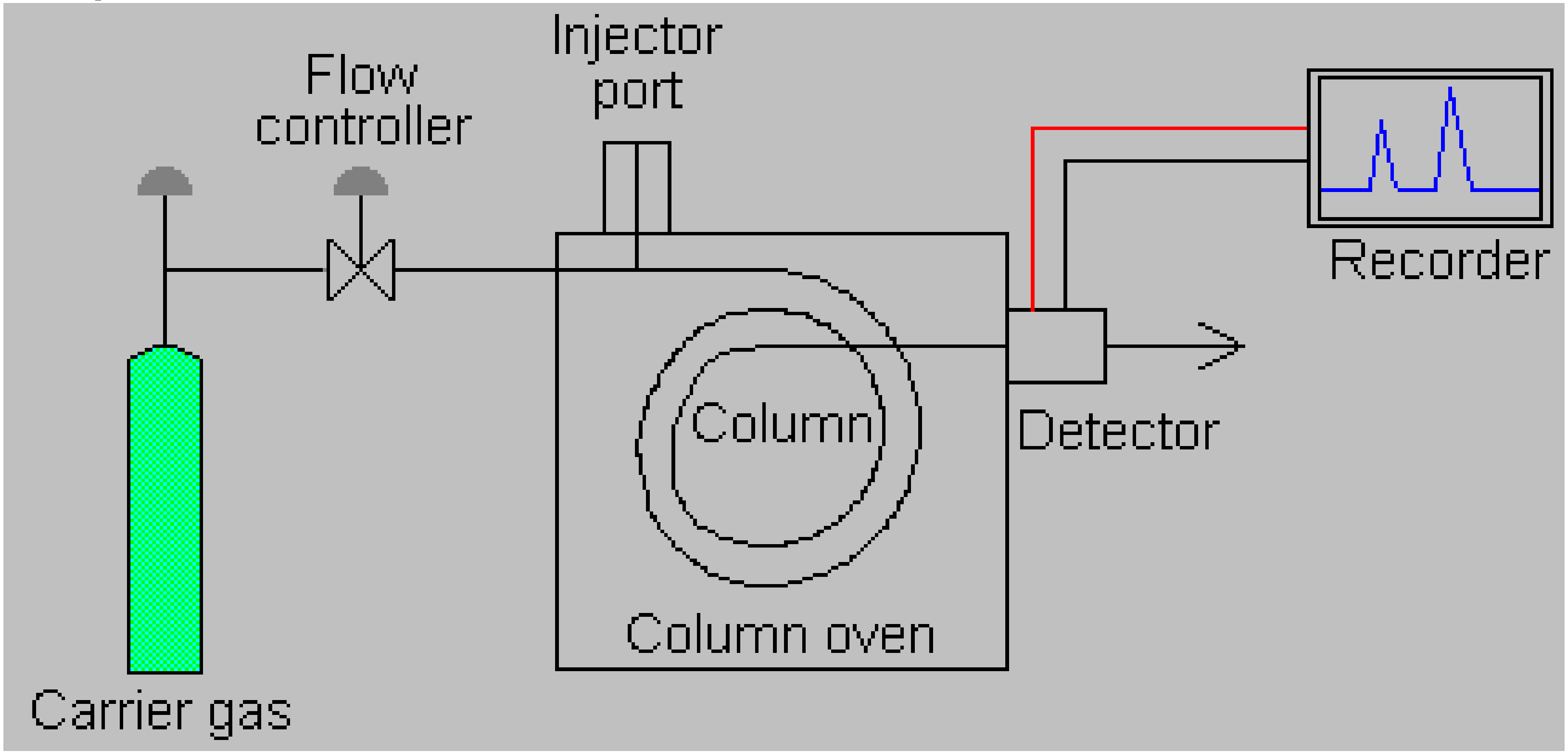
Poor resolution from poor efficiency



Poor resolution from poor selectivity

Schematic diagram of a gas chromatograph

1. Carrier gas
2. Sample injection port
3. Columns



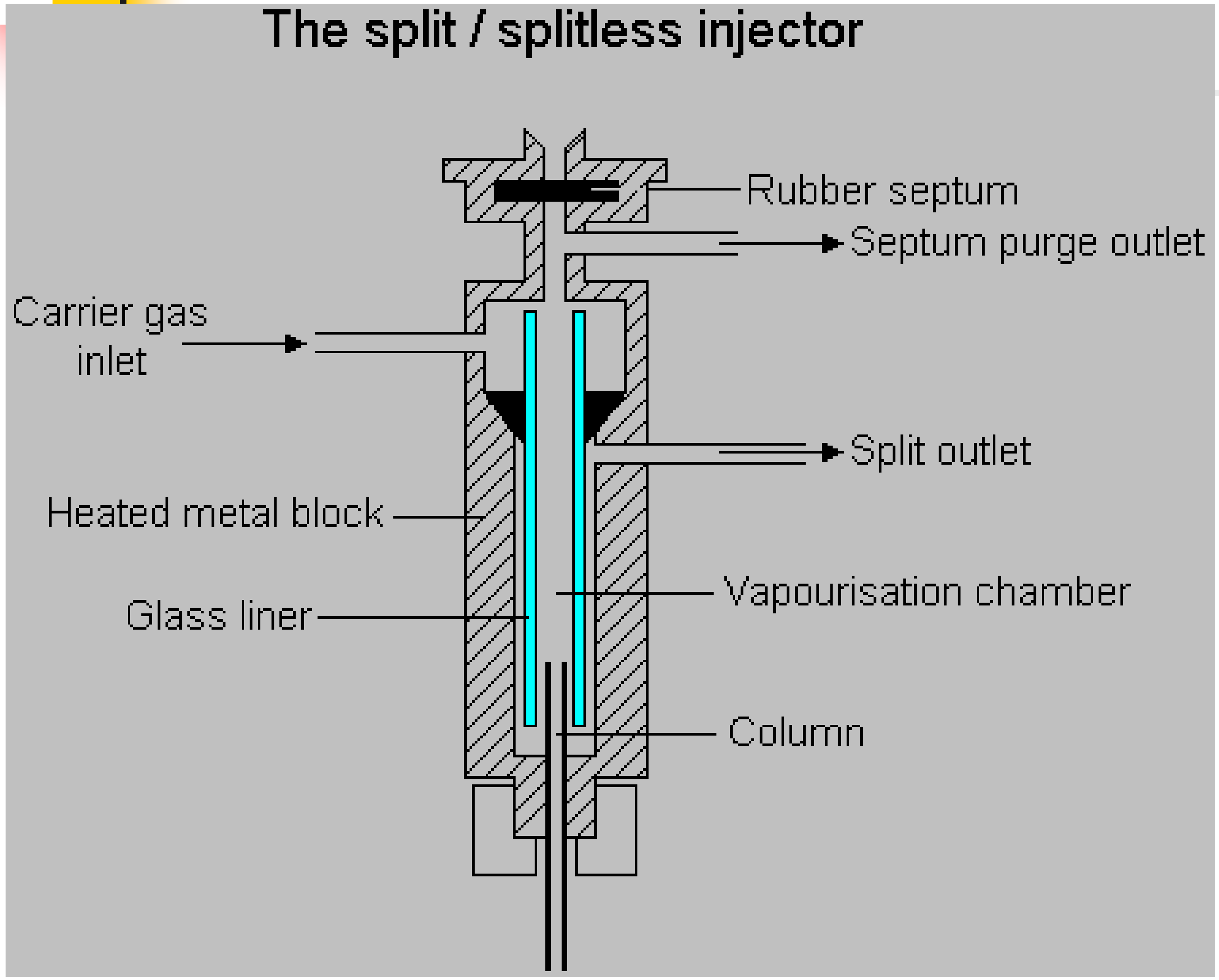
1. Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependant upon the type of detector which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities. Oxygen and hydrogen can't be used as they are active gases (not inert)

2. Sample injection port

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour - slow injection of large samples causes band broadening and loss of resolution. The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash vapouriser port at the head of the column. **The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample**. For capillary GC, **split/splitless** injection is used. Have a look at this diagram of a split/splitless injector;

2. Sample injection port: diagram of a split/splitless injector



Split or splitless.
(heated chamber) The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vapourises to form a mixture of carrier gas, vapourised solvent and vapourised solutes. **A proportion** of this mixture passes onto the column, but **most exits** through the split outlet. **The septum purge outlet prevents septum bleed components from entering the column.**

3. Columns

Packed columns contain a finely divided, inert, solid support material (commonly based on diatomaceous earth) coated with liquid stationary phase. Most packed columns are 1.5 - 10m in length and have an internal diameter of 2 - 4mm.

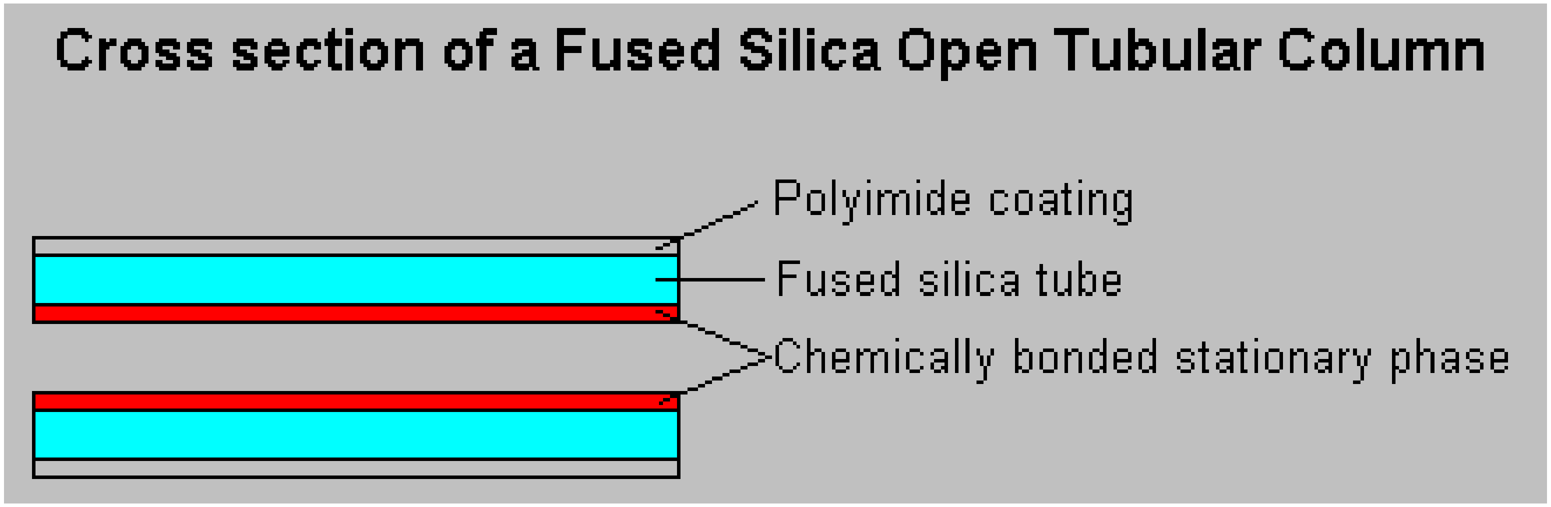
capillary (also known as open tubular) internal diameter of a few tenths of a millimeter.

Wall-coated open tubular (WCOT) consist of a capillary tube whose walls are coated with liquid stationary phase.

Support-coated open tubular (SCOT) the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed

generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

In 1979, a new type of WCOT column was devised - the Fused Silica Open Tubular (FSOT) column;



These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating. These columns are flexible and can be wound into coils. They have the advantages of physical strength, flexibility and low reactivity.

Commercially available stationary phases and equivalents

Cross-linked stationary phase	Commercial equivalent
Dimethylsiloxane	BP1, DB1, HP1, SE30,OV1, CPSil55%
Diphenyldimethylsiloxane	BP5, DB5, HP2, SE5414%
Cyanopropylphenyidimethylsiloxane	BP10, DB1701, OV170150%
Trifluoropropylmethylsiloxane	OV210, DB-210, QF-150%
Cyanopropylphenyidimethylsilaxane	BP225, OV225
Polyethylene glycol	BP20, DB-WAX, CW20M
Cyanopropylsilarylene	BPX70
Dimethylsiloxane-carborane copolymer	HT5
Dimethylsilarylene	BPX5

Selection of Stationary Phase

The key to good GC separations is to use the column with the most appropriate stationary phase.

A method to select the appropriate stationary phase for analysis of a sample mixture is to consider the polar characteristics of the analytes and select a stationary phase of similar polarity.

An analyte with similar polar character to the stationary phase **will be well retained**, the principle of like attracts like applies, and useful retention is then likely to occur leading to adequate selectivity and separation of the analytes primarily on the basis of volatility.

Types and classes of stationary phases

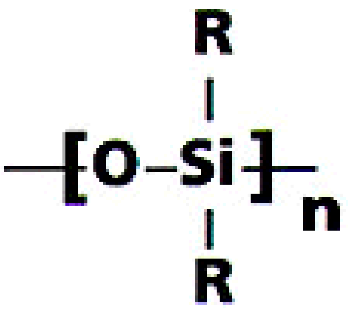
Polysiloxanes - Polyethylene Glycols - Gas-solid Stationary Phase

1. Polysiloxanes

Polysiloxanes are the most common stationary phases. They are the **most stable**, **robust** and **versatile**. Standard polysiloxanes are characterized by the repeating **siloxane backbone**. Each silicon atom contains two functional groups.

100 % methyl substituted such as DB-1 or HP-1.

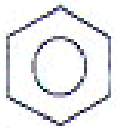
The phenyl group strengthens and stiffens the polymer backbone which inhibits stationary phase degradation at higher temperatures. This results in lower column bleed and, in most cases, higher temperature limits. **(e.g., DB-5).**



R = CH_3 methyl

$CH_2CH_2CH_2CN$ cyanopropyl

$CH_2CH_2CF_3$ trifluoropropyl

 phenyl

2. Polyethylene Glycols

$$HO[-CH_2-CH_2-O-]_nH$$

Polyethylene Glycol

<u>Polyethylene glycols (PEG)</u> are widely used as stationary phases .

They are <u>less stable</u>, <u>less robust</u> and <u>have lower temperature</u> limits than most <u>polysiloxanes.</u>

The unique separation properties of polyethylene glycol makes these liabilities tolerable. <u>Polyethylene glycol stationary phases must be liquids under GC temperature conditions.</u>

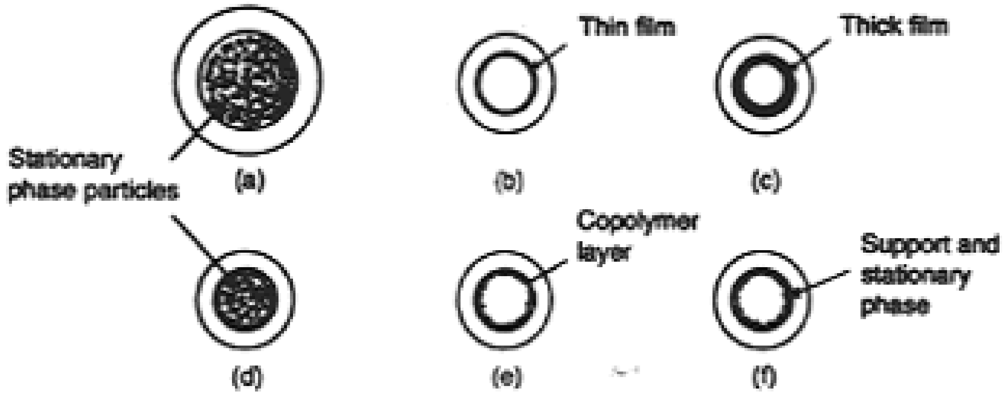
Exhibits better reproducibility and inertness (DB-WAX)

3. Gas-solid Stationary Phase

porous layer open tubular (PLOT) columns

thin layer (usually <10 mm) of small particles adhered to the surface of the tubing. The sample compounds undergo a gas-solid adsorption / desorption process with the stationary phase. The particles are porous, thus size exclusion and shape selectivity processes also occur. Various derivatives of styrene, aluminum oxides and molecular sieves are the most common PLOT column stationary phases.

Hydrocarbon and sulfur gases, noble and permanent gases, and low boiling point solvents are some of the more common compounds separated with PLOT columns.



Cross-section of GC columns
(a) 1/8 in. packed column;
(b) thin film WCOT column;
(c) thick film WCOT column;
(d) 1/16 in. micropacked column; (e) PLOT column;
(f) SCOT colum

Column temperature

For precise work, column temperature must be controlled to within tenths of a degree. The optimum column temperature is dependant upon the boiling point of the sample.

As a rule of thumb, a temperature slightly above the average boiling point of the sample results in an elution time of 2 - 30 minutes.

Minimal temperatures give good resolution, but increase elution times. If a sample has a wide boiling range, then temperature programming can be useful.

The column temperature **is** **isothermal** (constant temperature) or increased (**Linear programmed**, either continuously or in steps) or **Ramp programmed** (combined isothermal and linear programmed) as separation proceeds.

IV. Detectors

A non-selective: **detector responds to all compounds**

A selective detector: **responds to a range of compounds with a common physical or chemical property and a specific detector responds to a single chemical compound.**

Concentration dependant detectors: **the signal is related to the concentration of solute in the detector and does not usually destroy the sample**

Mass flow dependant detectors: **usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector.**

Detector √	Type √	Support gases	Selectivity √	Detectability	Dynamic range
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic cpds.	100 pg	10^7
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng	107
Electron capture (ECD)	Concentration	Make-up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50 fg	105
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10 pg	106
Flame photometric (FPD)	Mass flow	Hydrogen and air possibly oxygen	Sulphur phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100 pg	10^3
Photo-ionization (PID)	Concentration	Make-up	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics	2 pg	107
Hall electrolytic conductivity	Mass flow	Hydrogen, oxygen	Halide nitrogen, nitrosamine, sulphur		

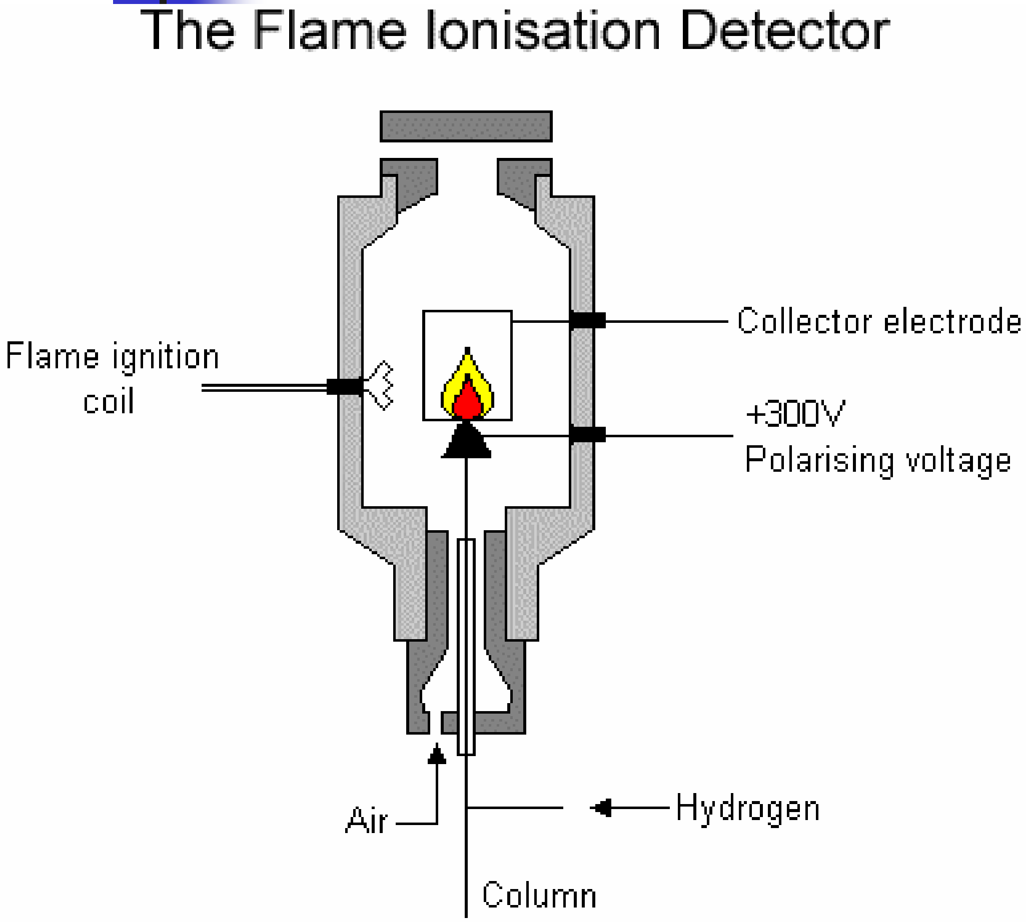
Minimum detection level (MDL)

MDL is the level of sample being measured by the detector at peak maximum (max concentration), when the detector signal (S), is at least twice the mean noise signal level (N)

$$S/N > 2 \quad (g\ ml^{-1})$$

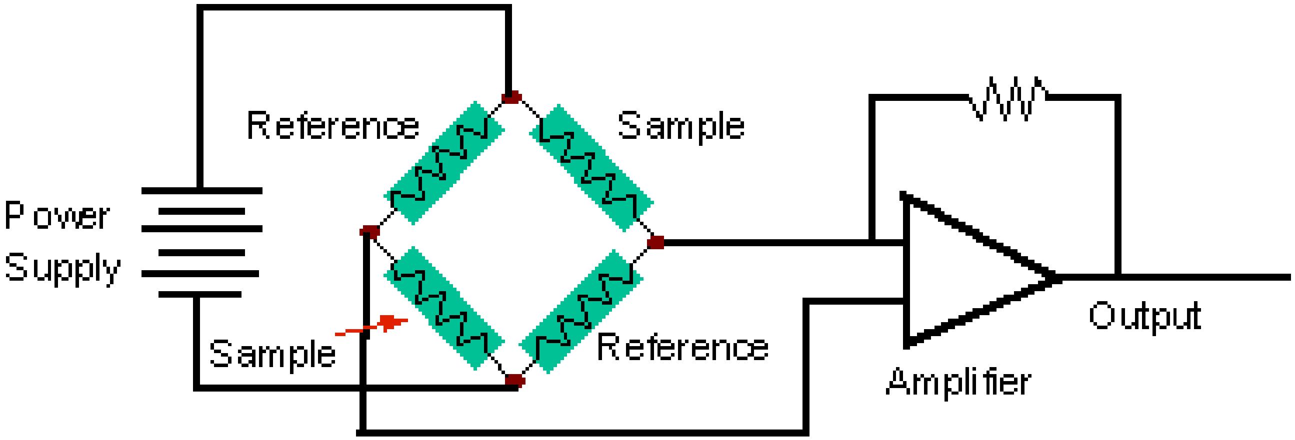
FID (10-12)

1. The flame ionisation detector FID



The effluent from the column is mixed with hydrogen and air, and ignited. Organic compounds burning in the flame produce ions and electrons which can conduct electricity through the flame. A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame. The FID is a useful general detector for the analysis of organic compounds; it has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but unfortunately, it destroys the sample.

2. Thermal Conductivity Detectors (TCD)



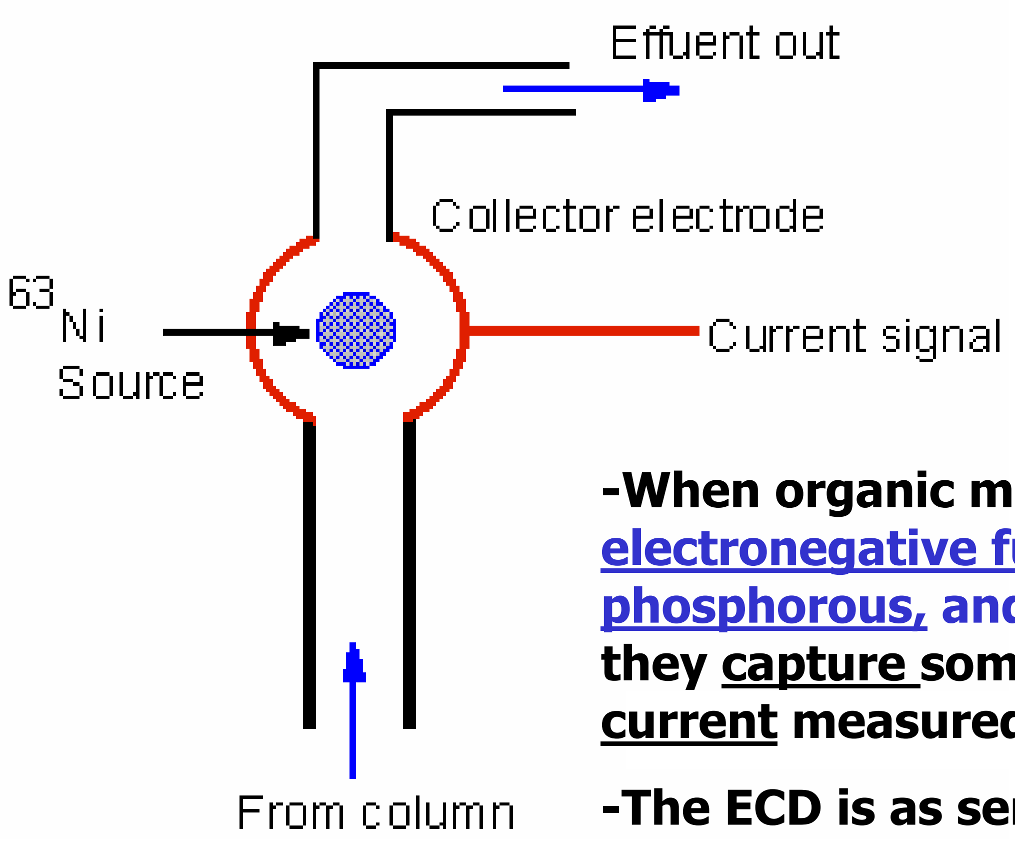
-A TCD detector consists of an electrically-heated wire or thermistor.

The temperature of the sensing element depends on the thermal conductivity of the gas flowing around it.

-Changes in thermal conductivity, such as when organic molecules displace some of the carrier gas, cause a temperature rise in the element which is sensed as a change in resistance.

-The TCD is not as sensitive as other detectors but it is non-specific and non-destructive.

3. Electron Capture Detectors (ECD)

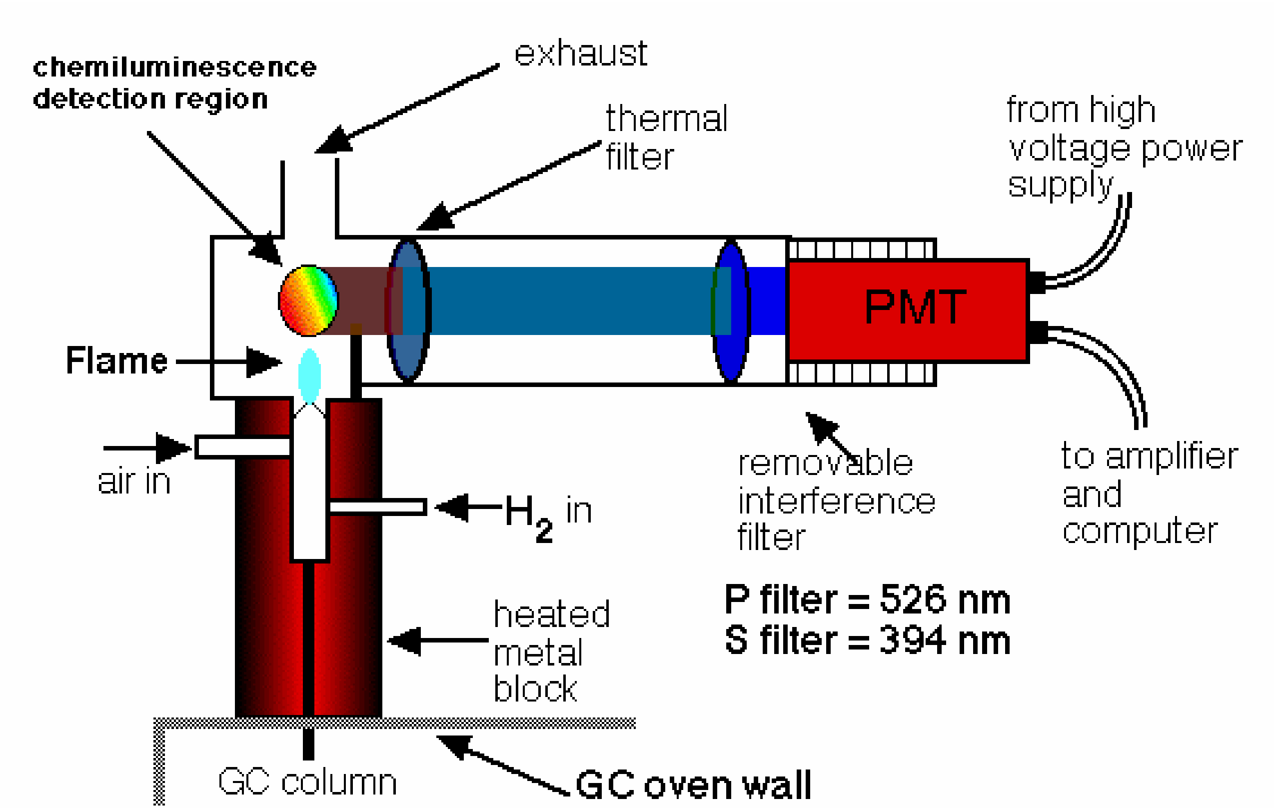


-The ECD uses a radioactive Beta emitter (electrons) to ionize some of the carrier gas and produce a current between a biased pair of electrodes.

-When organic molecules that contain electronegative functional groups, such as halogens, phosphorous, and nitro groups pass by the detector, they capture some of the electrons and reduce the current measured between the electrodes.

-The ECD is as sensitive as the FID but has a limited dynamic range and finds its greatest application in analysis of halogenated compounds.

4. Flame Photometric GC Detector

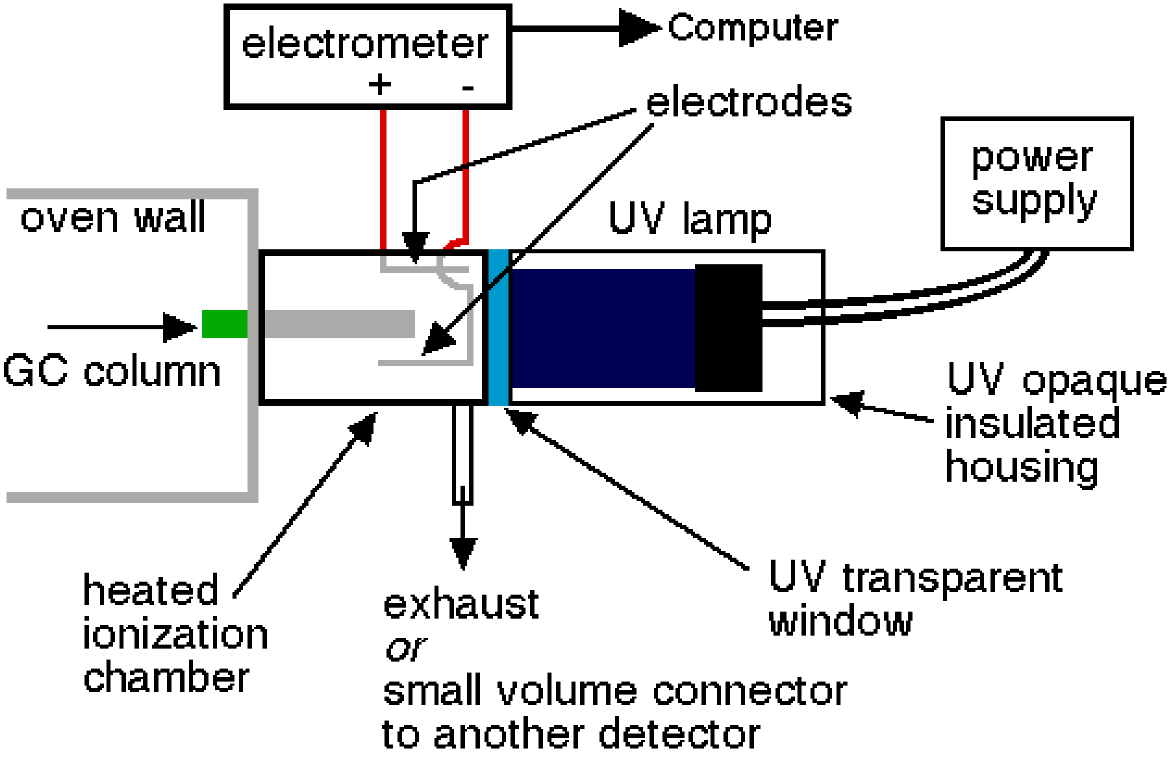


-The principle of operation concerns the measurements of **radiation** emitted by excited species in the flame

-In H_2 flame, **Sulphur** and **phosphorus** from excited species which emit radiation at 394 and 526 nm

-A photomulitiplier detects and amplifies the emissions to form the detector signal.

5. Photoionization Detector



-This device uses <u>ultraviolet light</u> as a means of ionizing an analyte exiting from a GC column.

-<u>The ions</u> produced by this process are collected by <u>electrodes</u>. The current generated is therefore a measure of the analyte concentration.

<u>A Photoionization Reaction</u>

$$R + h\nu \longrightarrow R^{+} + e^{-}$$

Sampling Techniques of Gas Chromatography

1. Derivatization of samples;

The derivatization of compounds for GC analysis is generally performed for a variety of reason;

•to increase volatility of the sample

•to reduce polarity of the compounds (acids, phenols, and some alcohols and amines)

•to reduce thermal degradation

•to increase detection response by introduction of functional groups (e.g. CF3) which are particularly sensitive to a selective detector such as ECD and NPD

•to extraction efficiency from aqueous media (e.g. acylation of phenolic amines)

Acylation

$$R_1-NH_2 \xrightarrow{(R_2CO)_2O} R_1-NH-C(=O)R_2 + R_2COOH$$

Alkylation

$$R_1-OH \xrightarrow{R_2X} R_1-O-R_2 + HX$$

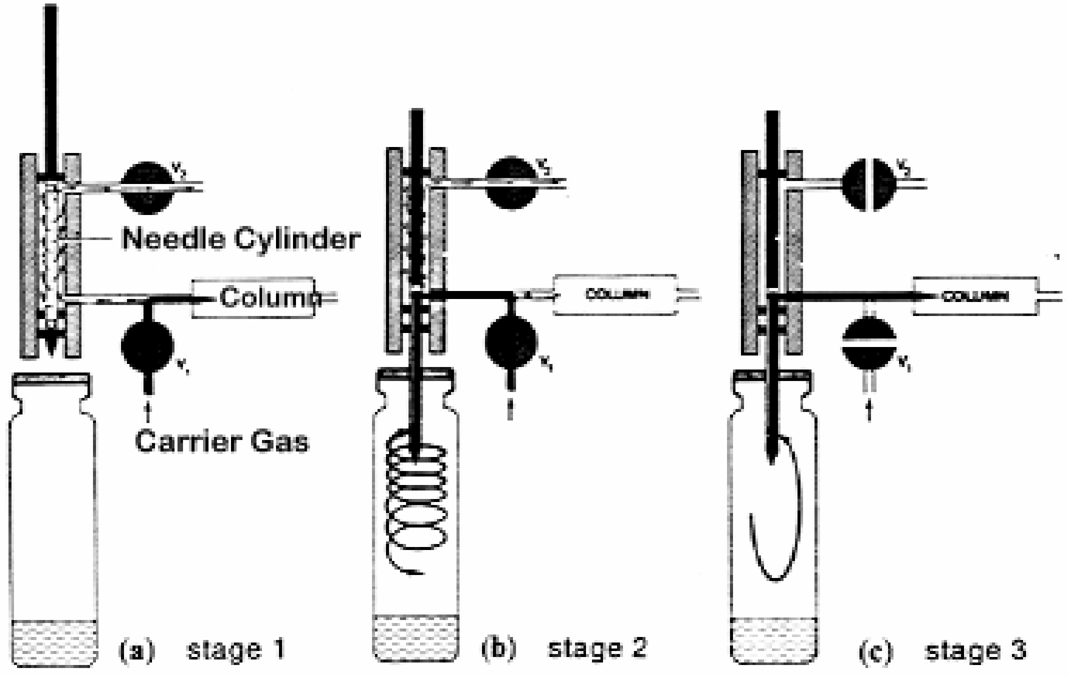
Silylation

$$R_1-OH \xrightarrow{(CH_3)_3SiX} R_1-O-Si(CH_3)_3 + HX$$

Condensation

$$R_1-NH_2 \xrightarrow{O=CR_2R_3} R_1-N=C(R_2)(R_3) + H_2O$$

2. Headspace sampling;

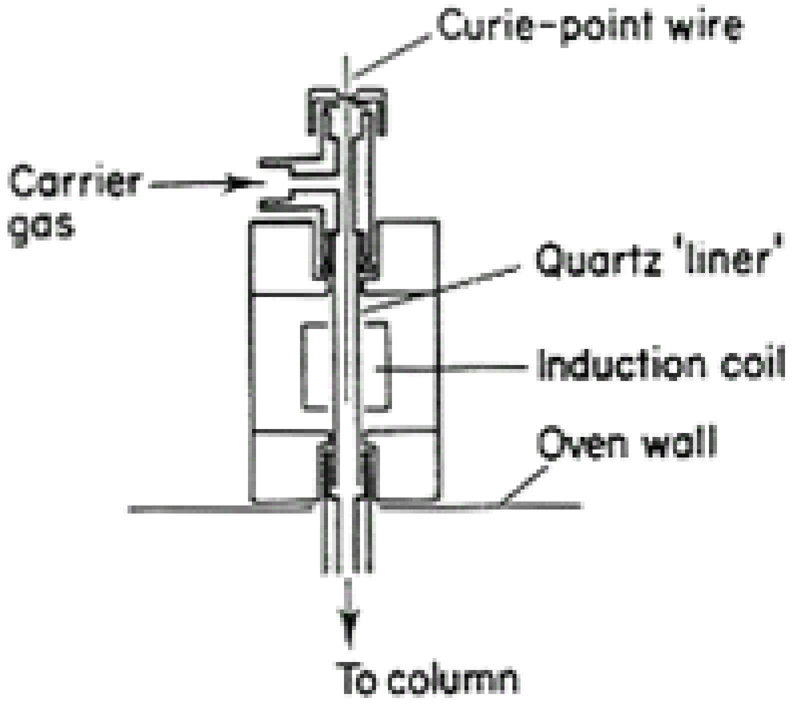


Static headspace sampling is the analysis of vapour above samples contained in a sealed vial.

Most volatile compounds in any matrix can be analyzed.

Sampling is achieved by transferring a predetermined volume of vapour phase into the column.

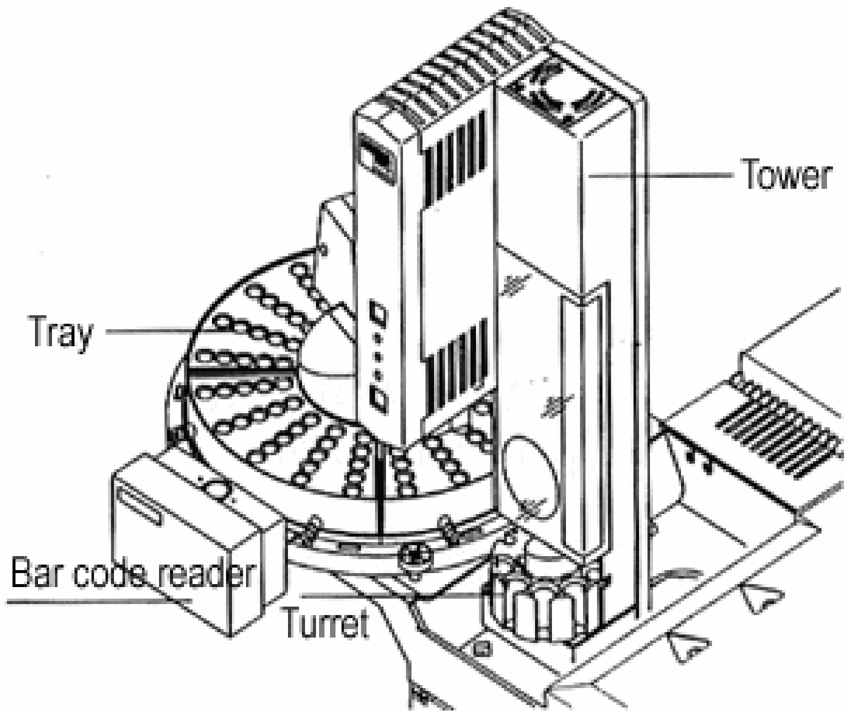
3. Sample introduction by pyrolysis;



A small amount of the sample is placed within a glass tube, which is then placed within a heater (curie point or ohmic heating), <u>and raised to a temperature of typically 1000 °C.</u>

This has the effect of breaking the material down into <u>lower molecular weight fragments which are carried by carrier gas (polymers and rubbers)</u>

4. Automated sample introduction;



Used in routine analysis.

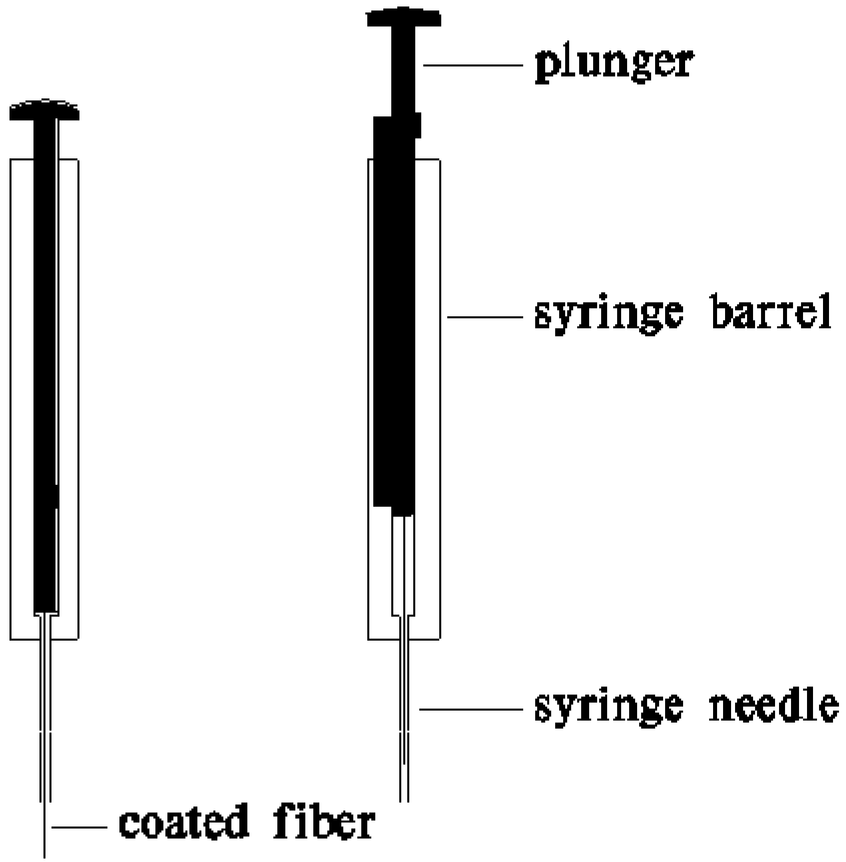
The entire process of sample transfer, sample injection, collection of chromatographic data and calculation of results is handled by computer.

5. Sampling by adsorption tubes;

Monitoring of the atmosphere and workplace for trace pollutants and compounds of environmental interest is now a common routine requirement to meet environmental legislation.

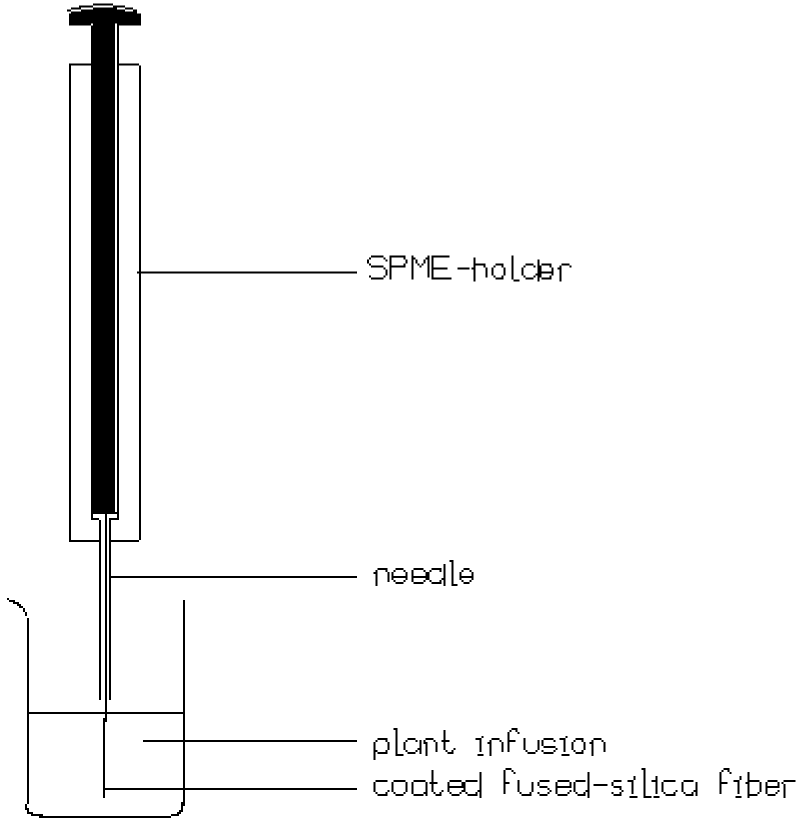
Substances to be analyzed are present at concentration levels down ppb, so is to collect the substances using an adsorbent tube.

6-Solid - Phase - Microextraction (SPME)

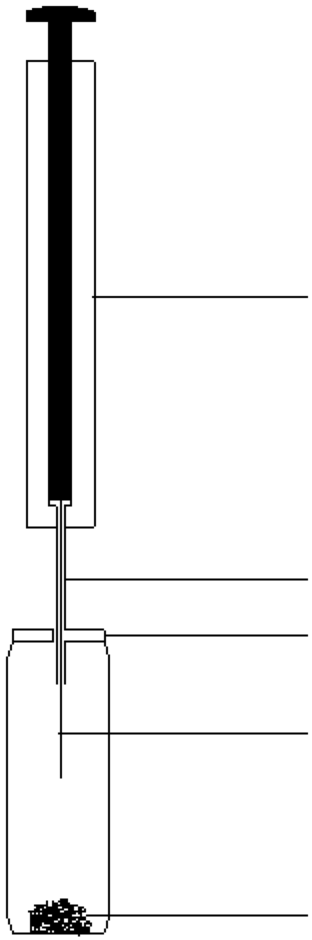


<u>Using polymer-coated fused silica fibres</u>. Sample preparation is based on the <u>adsorption</u> of analytes from a sample onto a coated fused silica fibre which is mounted in a modified GC syringe. After introducing the coated fibre into a liquid or gaseous sample, the compounds to be analysed are enriched according to their distribution coefficient and can be subsequently thermally desorbed from the coating after introducing the fibre into the hot injector of a gaschromatograph.

SPME-extraction procedure from a plant infusion



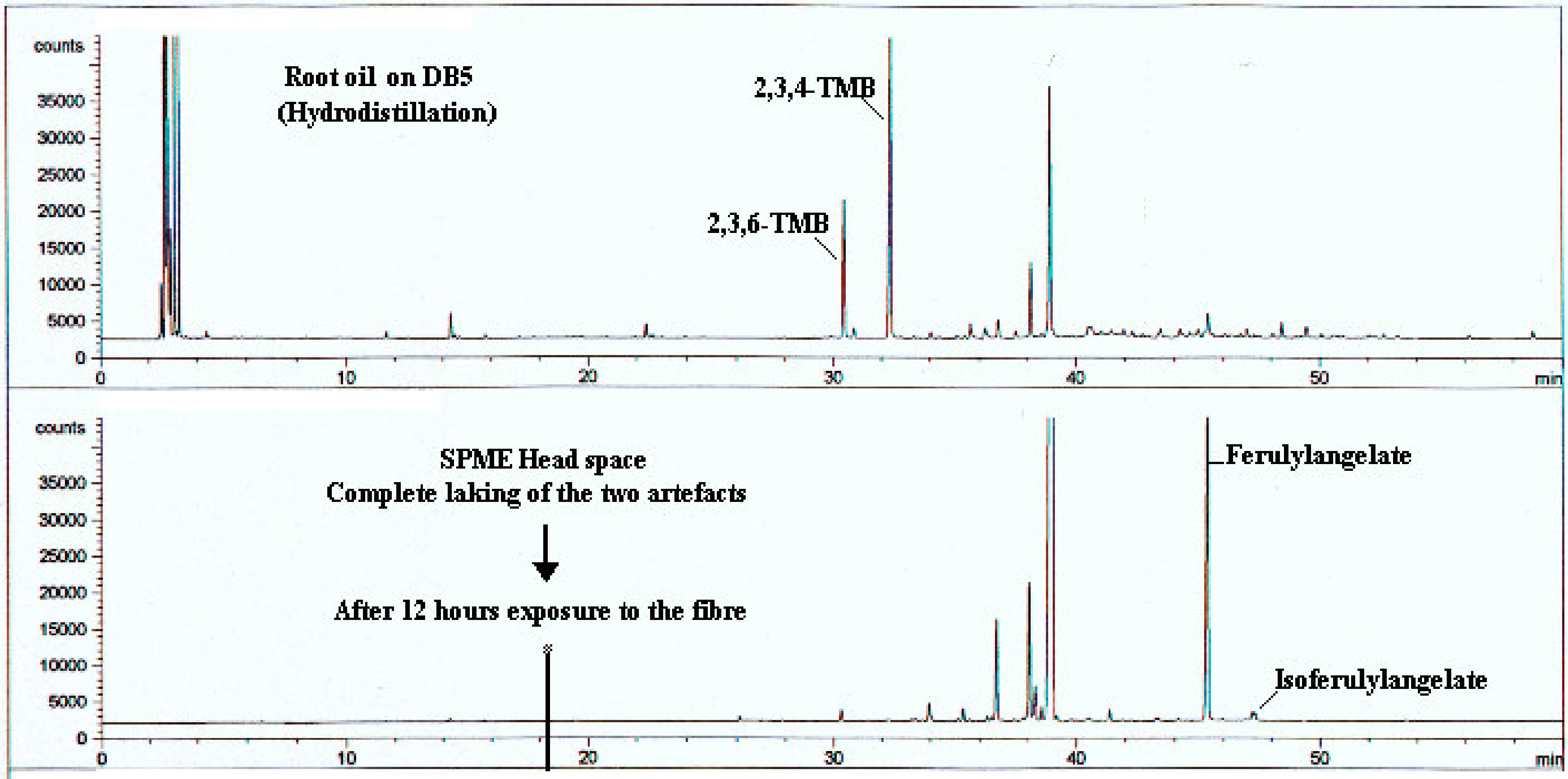
7-Headspace analysis by SPME.



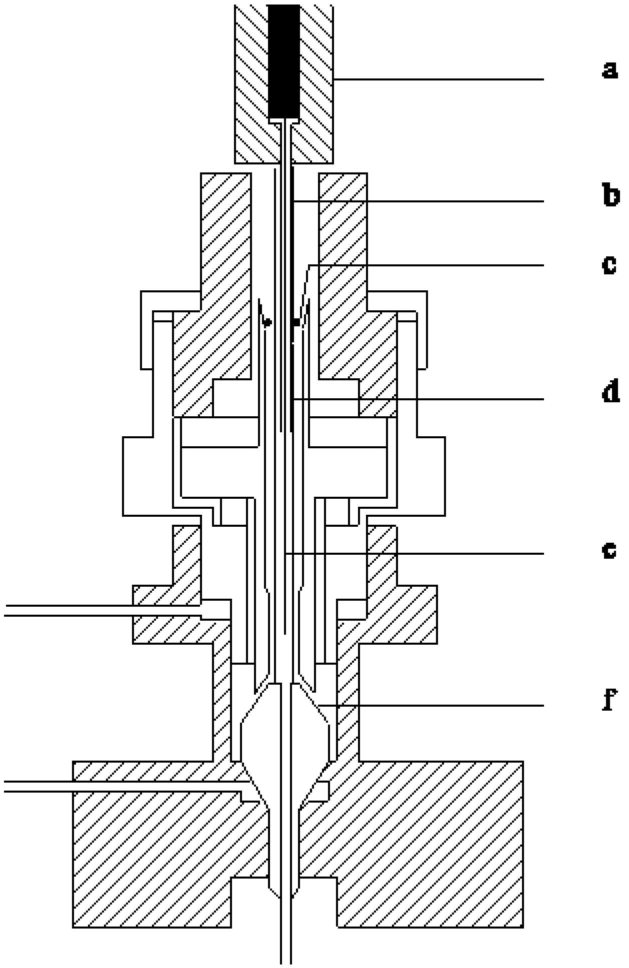
This analysis described by Kubeczka 1997 is applied by using coated fibers and SPME, respectively, refer to head space analysis as the following.

500 mg. sample of crushed plant material, which were placed in a 10 ml. crimp- cap glass vial with a teflon-lined rubber septum

Detection of artefacts by SPME (root oil of *Eryngium campestre*)



8-Peak transfer

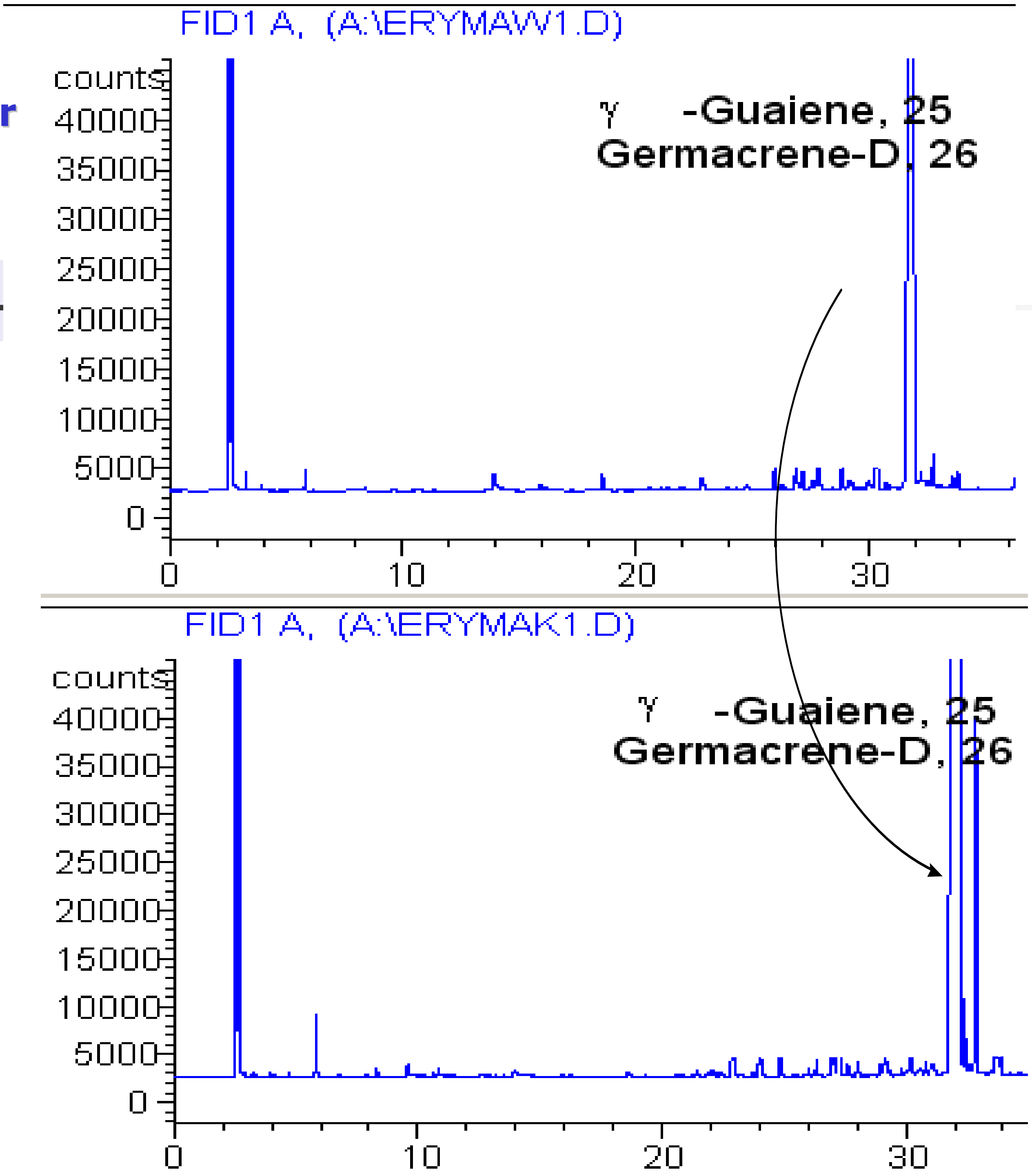


A new application described by Kubeczka 1997 of polymer coated fused silica fibres. This is a new approach of transferring fractions of capillary gas chromatographic run onto a second capillary column that means to perform a kind of column- switching or 2-dimensional gas chromatography.

This has to be done after a first normal GC.run yielding the exact information on retention times of the peaks to be transferred

At the beginning of peak- elution controlled only by time, a 100 µm Polydimethylsiloxane coated fibre was introduced into the mounted glass capillary tubing and withdrawn at the end of peak elution. Afterward, the fibre within the needle could be introduced into the injector of a second capillary column. e.g. with a different polar stationary phase.

8-Peak transfer



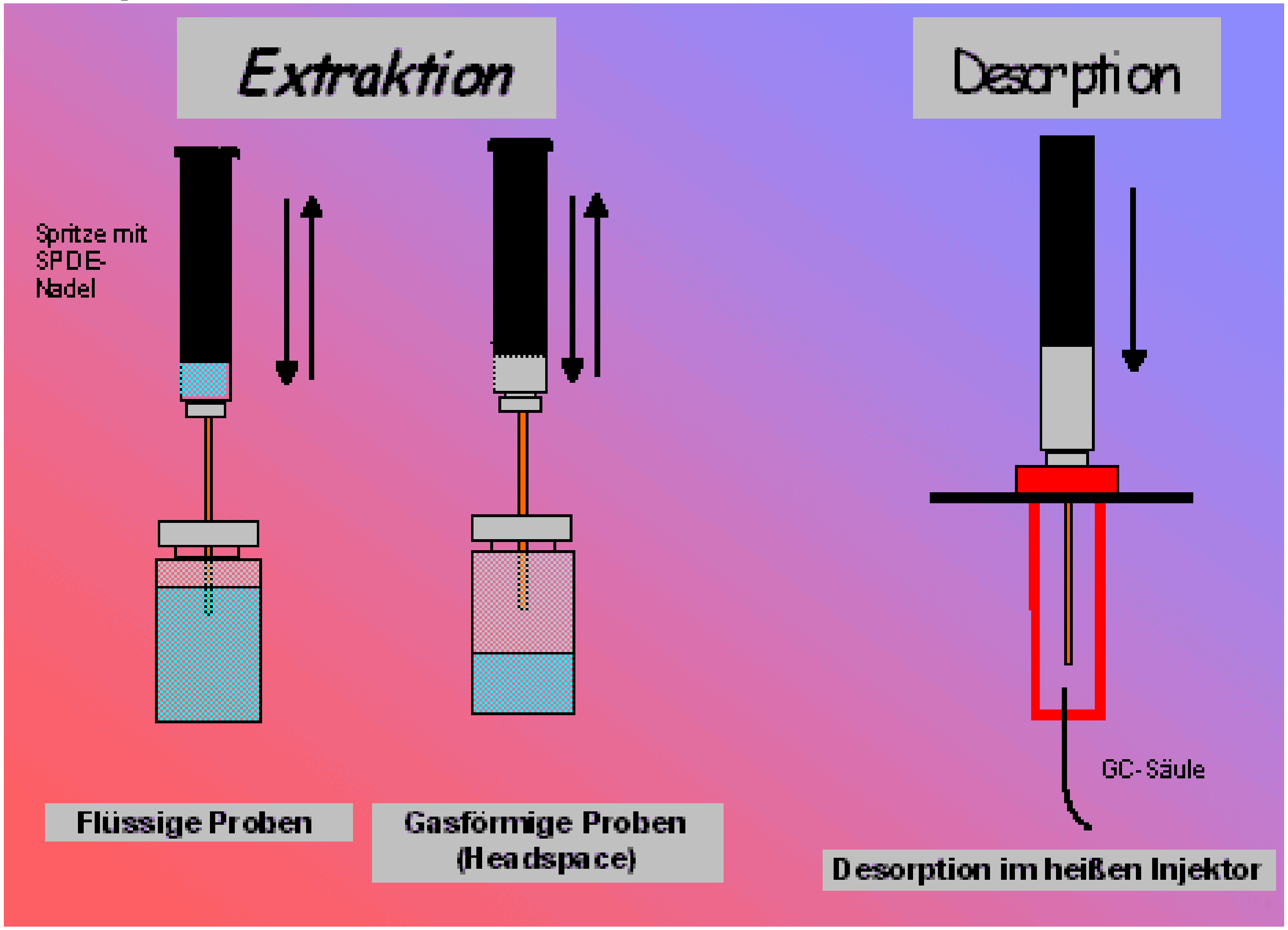
DB-5

(2 compounds overlaped under one peak)

DB-wax

(2 compounds are separated into 2 peaks)

9-Solid-Phase-Dynamic Extraction SPDE (The Magic Needle)



Principle: (advatages)

SPDE option makes the fully automatic dynamic sample extraction and analytic is possible. Liquid and Headspace samples can so substantially more simply and more reliably than with conventional SPME methods.

Description:

-A gastight syringe (developed in 2003 by Chromtech, Idstein, Germany) is equipped with a special needle coated on the inside with an extraction phase (stationary phase).

-A liquid or head-space (gaseous or HS-SPDE) sample is drawn up into the needle, adsorbing analytes onto the stationary phase. A distribution balance is reached between the liquid or gas sample matrice and the phase.

-The analytes are concentrated onto the phase by repeatingly introducing the sample through the needle. The analytes are desorped from the syringe needle when introduced into a hot injector of a gaschromatograph.

-The vaporizing components are transferred onto the injector and separation column using carrier gas passing through the inlet side whole of the syringe and onto the needle. This followed by gas chromatography analysis. The usual phases (PDMS, Carbowax, Divinylbenzene) are available.

9-Solid-Phase-Dynamic Extraction

Application of GC

Head Space GC

Forensic analysis of volatile compounds (ethanol, actone….) in blood and urine. The blood sample is homogenized, a part is diluted with aqueous solution of internal standard (*n*-propanol or isopropanol). Aqueous solution of ethanol is used as standard to calibrate the internal standard. The sealed vials containing the diluted samples are allowed to equilibrate at 40°C for 10 mint., the vapour samples are then analysed on polar column such as PEG 400 or Porapak Q.

Detection of volatiles such as white spirits, paraffin, petrol, diesel fluid and glue solvents in the blood may help the determination of cause of death in arson, explosion and accident cases.

HSGC, analysis of aromatic flavours, trace volatile in beer, foods and soft drinks.

Plastic packing and consumer products are analysed for residual monomers and other residues such as O_2, CO_2, H_2O, actates

Analysis of volatile fatty acids produced by bacteria specially the anaerobic bacteria, assists in the identification of the bacteria.

Fats and oils

Free fatty acids (FFA) and fatty acid methyl esters (FAME)

- Glass column gives the best results for volatile FA
- The preferred method for quantitative analysis of FAs is to first convert them to ME esters (metal column is suitable)

Butyl esters are preferred for the lower molecular weight acids

Halognated esters can be made for enhanced sensitivity with ECD

Saturated FAME: non polar stationary phase

Unsaturated FAME: polar stationary phase

- Dibasic acids, Alkylation gives dialkylesters, then run on polar column
- Hydroxy acids, run on capillary column as they are active and labile.

Proteins

On hydrolysis gives amino acids, which on esterification resulting in volatile compounds ready for GC.

Amines

Very polar, basic required priming with basic metal e.g. KOH to avoid tailing. Glass column is also recommended to avoid tailing and derivatize by silylation or acylation.

Hydrocarbons

C1-C5 (packed, metal column)

> C6 (capillary column gives high resolution)

Alcohol and phenol

Should be silylated or acylated.

Carbohydrates

<u>Sugars, mucilage, pectin, cellulose</u>

Monosacchreids, silylation or alditol acetates.

Di and polysacchreids, should be first hydrolysed to liberate the hemiacetal included in chain formation, then silylation to give volatile derivative then GC on glass column. The TMS derivatives run only on column using stationary phase free from active H e.g. Silicones (OV-1,DB-1)

Flavours and fragrances

Analysis of essential oils: Flavours and fragrances are complicated mixtures made up of pure essential oils or mixture of them. Natural flavours and fragrances are more complex than synthetic mixtures, so capillary column are preferred. They are complicated mixtures of terpenes (mono and sesquiterpenes, as hydrocarbons or oxygenated compounds). The odour of volatile oils are due to the oxygenated compounds (polar), while the non-oxygenated hydrocarbons have no characteristic odour and non polar.

The analysis of complicated mixture of essential oil

Preparation of the sample for analysis: If V.O. is present in high concentration, then apply **steam distillation**. In case of low concentration **solvent extraction** is preferred (V.O. from flowers). **Expression method** is the method of choice for the isolation of V.O. from the fruits. For aromatic water e.g. lemon water, rose water, **salting out** by NaCl is applied to separate V.O. from water, followed by light organic solvent extraction. Headspace could be also used for such analysis.

Fractionation of the sample: applying silica gel column, then elution with different solvents of different polarities.

Hexane to separate the non polar fraction

Ether to separate the medium polar fraction

Methanol or ethanol to separate the polar fraction

Evaporate the solvents, individually, at low temperature under reduced pressure to a concentration suitable for GC analysis using stationary phase with the same polarity.

Pesticides

(insecticides, fungicides, herbicides, rodenticides and nematocides)

Chlorinated pesticides: packing DB1(ECD)

Carbamates and thiocarbamates: they are thermally unstable, so column and supports must be deactivated (silylated). Packing (5% DB1)

Organo-P-Pesticides: they may contain S group, packing (DB1)), detector (FPD)

Polychlrorinated Biphenyls (PCB's): They are not pesticides but associated with pesticide samples. They are used as transformer oils plasiticizers or as GC stationary phase. Column (capillary column), packing (1% dextrose 300) and detector (ECD).

Food analysis:

- Lipids – hydrolysis - derivatization to fatty acid methyl esters - GC
- Proteins - acid hydrolysis - amino acids - esterification to n-propyl esters-GC
- Carbohydrates – hydrolysis – silylation – GC. (if monosaccharides, no hydrolysis).
- Fruits, fruit-derived food, vegetables, soft drinks, beverage (tee and coffee) are analysed for their polybasic acid content or hydrolysis acid content (e.g. citric, maleic acids,) as TMS derivatives using an OV-17 column.
- Flavanols and caffeine are analysed on DB1
- Carbonyl-containing volatiles e.g. are analysed using carbowax (polar column).

GC used in analysis of meat: GC can be used to determine the origin of meat and differentiate between them, This can be achieved by determinig the ratio between certain fatty acids e.g.

Pork fat (pure lard) **C16 / C14 or C18:1 / C14** > **6**

C16: palmitic acid, C14 myrestic acid, C18:1 oleic acid.

Beef and lamb: < 4 Duck fat: > 14

Drugs

Forensic analysis uses GC to characterise drugs of abuse in tissue and fluids

GC for salts of basic compounds (e.g. amine HCl or SO4) fail because of irreproducibility breakdown to the free amine or because of elongated peak tail, so difficult to be quantitated. This can be avoided by extraction of the free base after neutralisation or direct derivatization of the salt. e.g. determination of opium alkaloids in pharmaceutical preparation. When GC is applied for free steroids and alkaloids content, NH2 group shows tailing, bad resolution and low sensitivity, this can be overcomed by derivatization.

GC of basic N-compounds e.g. Alkaloid shows adsorption problem, which can be eliminated by incorporating basic material (KOH) into the support or use highly silanized support, or both.

N-Hexylamine can be added to the carrier gas to facilitate the analysis of non volatile N-compounds.

For non volatile pharmaceutical products not easily derivatized, pyrolysis GC is applied e.g. for antibiotics (penicillin and cephalosporins).

Some drugs can be analysed directly after extraction or dissolution in solvent, while others required derivatization.. <u>Retention data is available for over 600 drugs, this is useful for forensic analysis to assist identifying abused drugs</u>

The best ASU 2008 gradutes we all had ever seen

Finally ThanK You Will miss U Love U all

Wish u all the best

Nahla Ayoub